

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Eijiro WATANABE et al.

Serial No.: 08/992,914

Group: 1638

Filed: December 18, 1997

Examiner: D.H.Kruse

For: RAFFINOSE SYNTHASE GENES AND THEIR USE

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Akitsu NAGASAWA, citizen of Japan and residing in Kamokogahara 3-28-56, Higashi-Nada-ku, Kobe-shi, Hyogo-ken, Japan, declare and say that:

1. I completed the master's course, with a major in agricultural biology, of the graduate school of Kyoto University and obtained a master's degree in agriculture at Kyoto University in March, 1984.

2. From April, 1984 to the present, I have been an employee of Sumitomo Chemical Company, Limited, the assignee of the above-identified application.

3. From April, 1984 to the present, I have been engaged in research works for plant engineering using recombination and other gene manipulation, such as cloning of plant genes, preparation and evaluation of transgenic plants.

4. I am one of the members of the research project related to the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action mailed March 11, 2005 and the reference cited, and am familiar with the subject matter thereof.

6. To demonstrate successful identification of raffinose synthase genes in plant, I have made the following computer analysis.

ANALYSIS

1) The overall sequence homologies (%) among the amino acid sequences of raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) shown in Table 1 attached hereto were calculated based on a global multiple alignment (the alignment of sequences over their entire length) using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. The global multiple alignment was generated using CLUSTAL sequence analysis program. The amino acid sequences of the RFSs, SIP and STSs used to produce the global multiple alignment are as follows:

Sc-02:

MAPPSITKTATLQDVISTIDIGNGNPLFSITLDQSRDFLANGHPFLTQV
PPNITTNTTASSFLNLKSNKDTIPNNNNTMLLQQGCFVGFNSTEPKSH
HVVPLGKLKGIKFMSIFRFKVWWTTHWVGTFNGQELQHETQMLILDKNDSL
GRPYVLLLPILENTFRDSLQPGLNDHIGMSVESGSTHVTGSSFKACLYIH
LSNDPYSILKEAVKVIQTQLGTFKTLEEKTAPSIIDKFGWCTWDAYLKV
HPKGVWEGVKSLTDGGCPGPFIIDDGWQSICHDDDDEDDSGMNRTSAGE
QMPCRLVKYEENSFKFREYENPENGGKKGLGGFVRDLKEEFGSVESVYWH
ALCGYWGGVRPGVHGMPKARVVVPKVSQGLKMTMEDLAVDKIVENGVGLV
PPDFAHEMFDFGLHSHLESAGIDGVKVDVIHLLELLSEYGRVELARAYY
KALTSSVKKHFKGNGVIASMEHCNDFFLLGTEAISLGRVGDDFWCSDPG
DPNGTYWLQGCHMVHCAYNSLWMGNFIQPDWDMFQSTHPCAEFHAASRAI
SGGPIVYSDCVGNHNFKLLKSLVLPDGSILRCQHYALPTRDCLFEDPLHN
GKTMLKIWNLNKYTGVLGLFNCQGGGWCPEARRNKSVSEFSRAVTCYASP
EDIEWCNGKTPMSTKGVDFFAVYFFKEKKLRLMKCSDRLKVSLEPFSFEL
MTVSPVKVFSKRFIQFAPIGLVNMLNSGGAIQSLEFDDNASLVKIGVRGC
GEMSVFASEKPVCCKIDGVVKVFLYEDKMARVQILWPSSSTLSLVQFLF

Sc-03:

MAPSFSKENSKTDEVANHDCNTCPISLEESNFMVNNGHVILSQVPSNI
TAISKMGFDGLFVGFDAPEPKARHVSVGQLKGIPFMSIFRFKVWWTTHW
TGSNGRDLEHETQILILDKSDEGLGRPYIVILPLIEGPFRASLQPGSVDD
YVDICVESGSTKVGDSFRAVLYIRAGPDFKLIKDTMKEVQAHLGTFKL
LDDKTPPGIVDKFGWCTWDAYLKVEXYGVWEGVKGLVENGVPPGLVILD
DGWQSICHDDDPITDQEGINRTSAGEQMPCRLIKYEENFKFRDYKSPNIM
GHEDHPNMGMRAFVRDLKEEFKTVEHVVYVHAFTGYWGGVRPNVPGLEXA
QVVTPLSPGLEMTMEDLAVDKIVNNGIGLVQPDKAQELYEGLHSHLENC

GIDGVKVDV IHLLEMMAEDYGGRVELAKTYYKAI TESVRKHFKGNGVI AS
MEQCNDMFMLLGTE I CLGRVGDDFWPTDPSGDINGTYWLQGCHMVHCAYN
SLWMGNF IHPDWDMFQSTHPCAEFHAASRAISGGPIYVSDVVGKHNIPLL
KRLVLADGSILRCEYHALPTKDCFLVDPLHDGKMLKIWNLNKYNGVLGV
FNCQGGGWSRESRKNLCFSEYSKPISCKTSPKDVEWENGHKPFPKGVEC
FAMYFTKEKKILSQLSDTIEISLDPFDYELIVVSPMTILPWESIAFAPI
GLVNMLNAGGAVKSLDISEDNEDKMQVQVGIKGAGEMMVYSSSEKPKACRVN
GEDMEFEYEESMIKVQVTWNHNSGGFTTVEYLF

Sc-04 (truncated) :

MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPLTEVPENIIVT
PSPIDAKSSKNNEDDVVGCFVGFADEPRSRHVASLGKLRGIKFMSIFR
FKVWWTTHWVGSGNGHELEHETQMMLLDKNDQLGRPVLILPILQASFRAS
LQPGDDYVDVCMESGSTRVCSSFGSCLYVHVCHDPYQLLREATKVRM
HLGTFKLLEEKTAPEVIIDKFGWCTWDAYLKVHPSGVWEGVKGLVEGGCP
PGMVLIDDGWQAI CHDEDPITDQEGMKRTSAGEQMPCCRVLKLEENYKFRQ
YCSGKDSEKGGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ
AKVVTPLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLYEGLHSRLES
AGIDGVKVDV IHLLEMSEEYGGRVELAKAYYKALTASVKKHFKGNGVI A
SMEHCNDFFLLGTEAI ALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY
NSLWMGNF I QPDWDMFQSTHPCAEFHAPLGPSLVDQFTLVIVLESTSSC
SRASLCLMGRFCVVNTMHSPHETVCLKTPCMMGRQCSKFGISTNIQVFWV
YLIAKEVGGVP

Sc-05 :

MAPPSVIKSDAAVNGIDL SGKPLFRLEGS DLLANGHVLT DVPVNVTVA
SPYLA DKDGE PV DASAGSF IGFNLDGE PRSRHVA SIKL RDIFRMSIFR
KVWWTTHWVGSKGSDIENETQI I ILENSGSGRPYVLLLPLLEGSFRSSFQ
PGEDDDAVCVESGSTQVTGSEFRQVYVHAGDDPFKLVKDAMKVVRVHM
NTFKLLEEKXPPGIVDKFGWCTWDAYLTVNPDGVHKGVKCLVDGGCPPG
LVLIDDGWQSIGHDSGIDVEGMSCTVAGEQMPCCRLLKFQENFKFRDYVS
PKDKNEVGMKAFVRDLKEEFSTVDYIYVWHALCGYWGLRPGAPTLPPST
IVRPELSPGLKLTMQDLAVDKIVDTGIGFVSPDMANEFYEGLHSHLQNVG
IDGVKVDV IHLLEMCEKYGGRVDLAKAYFKALTSSVNKHFDGNGVI ASM
EHCNDMFMLGTEAI SLGRVGDDFWCTDPSGDI NGTYWLQGCHMVHCAYNS
LWMGNF I QPDWDMFQSTHPCAEFHAASRAISGGPIYISDCVGQHDFDLLK
RLVLPDGSILRCEHYALPTRDRLFEDPLHDGKMLKIWNLNKYTGIIGAF
NCQGGGWCETRRNQCF SQCVNTLTATTNPKDVEWNSGNNPISVENVEEF
ALFLSQSKKLVLSGPNDDLEITLEPFKFELITVSPVVTIEGSSVQFAPIG
LVNMLNTSGAIRSLVYHEESVEIGVRGAGEFRVYASRKPA SKIDGEVVE
FGYEE SVMVQVPWSAPEGLSSIKYEF

PsRFS :

MAPPSITKTATQQDVISTVDIGNSPLLSISLDQSRNFLVNGHPFLTQVPP
NITTTTSTPSPLDFKSNKDTIANNNTLQQQGCFVGFTNTEAKSHVV
PLGKLKGIKFTSIFRFKVWWTTHWVGNTNGHELQHETQILILDKNISLGRP
YVLLPILENSFRSLQPGLNDYVDMVESGSHVTGSTFKACLYLHLSN
DPYRLVKEAVKVIQTKLGTFTLEEKTPPSIEKFGWCTWDAFYLKVHPK
GVWEGVKALTDDGCPPGFVIIDDGWQSISHDDDPVTERDGMNRTSAGEQ
MPCRLIKYEENYKFREYENGDNGGKGLVGFVRDLKEEFRSVEVYVWHA
LCGYWGGRPKVCGMPEAKVVVPKLSPGVKMTMEDLAVDKIVENGVGLVP
PNLAQEMFDGIHSHLESAGIDGVKVDVIHLLELLSEYGGRVELAKAYYK
ALTSSVNKHFKGNGVIASMEHCNDFFLLGTEAISLGRVGDDFWCCDPSGD
PNGTYWLQGCHMVHCAYNSLWMGNFIHPDWDMFQSTHPCAEFHAASRAIS
GGPVYVSDCVGNHNFKLLKSFVLPDGSLRQCQHYALPTRDCLFEDPLHNG
KTMKCIWNLNKYAGVLGLFNCQGGGWCETRRNKSASEFSHAVTCYASPE
DIEWCNGKTPMDIKGVDVFAVYFFKEKKLSLMKCSDRLEVSLEPFSFELM
TVSPLKVF SKRLIQFAPIGLVNMLNSGGAVQSLEFDDASLVKIGVRGCG
ELSVFASEKPVCCKIDGVSVF DYEDKMVRVQILWPGSSTLSLVEFLF

Aj-05 :

MAPSFKNNGGSNVVSDGLNDMSSPFAIDGSDFTVNGHSFLSDVPENIVAS
PSPYTSIDKSPVSVGCFVGF DASEPDSRHVVSI GKLKDIFRMSIFRFKVW
WTTHWVGRNGGDLESETQIVILEKSDSGRPYVFLPIVEGPFRTSIQPGD
DDFVDVCVESGSSKVVDASFRSMLYLHAGDDPFALVKEAMKIVRTHLGT
RLLEEKTPPGIVDKFGWCTWDAFYLTVHPQGVIEGVRHLVDGGCPPGLVL
IDDGWQSIGHDSDPITKEGMNQTVAGEQMP CRLKQFQENYKFRDYVNPKA
TGPRAGQKGMKAFIDELKGEFKTVEHVYVWHALCGYWGLRPQVPGLEA
RVIQPVLSPGLQMTMEDLAVDKIVLHKVGLVPPEKAEMYEGLHAHLEKV
GIDGVKIDVIHLLEMLCEDYGGRVDLAKAYYKAMTKSINKHFKGNGVIAS
MEHCNDMFLGTEAISLGRVGDDFWCTDPSGDPNGTFWLQGCHMVHCAND
SLWMGNFIHPDWDMFQSTHPCAAFHAASRAISGGPIYVSDSVGKHNF DLL
KKLVLPDGSLRSEYYALPTRDCLFEDPLHNGETMLKIWNLNKFTGVIGA
FNCQGGGWCETRRNQCSQYSKRVTSKTNPKDIEWHSGENPISIEGVKT
FALYLYQAKKILSKPSQDLDIALDPFEFELITVSPVTKLIQTSLHFAP
GLVNMLNTSGAIQSVDYDDDLSSVEIGVKGC GEMRVFASKPRACRIDGE
DVGFKYDQDQM VVQWPWIDSSGGISVIEYLF

HvSIP :

MTVTPQITVGDGRLAVRGRTVLSGVPDNVTAHAAGAGLVDGAFVGATAA
EAKSHHVFTFGTLRDCRFMCLFRKLWWMTQRMGTSGRDVPLETQFILIE

VPAAAGNDDGDSSDGDSEPVLYMLPLLEGQFRVLQGNDQDELQICIES
GDKAVETEQGMNNVYVHAGTNPFDTITQAVKAVEKHTQTFHHREKKTVP
FVDWFGWCTWDAYTDVTADGVKQGLRSLAEGGAPRFLIIDDGWQQIGS
ENKDDPGVAVQEGAQFASRLTGIRENTKFQSEHNQEETPGLKRLVDET
EHGVKSYYVWHAMAGYGGVKSAGMEHYEPALAYPVQSPGVTGNQPD
VMDSLSVLGLGLVHPRRVHRYDELHAYLAACGVGVKVDVQNI
VETLGA
GHGGRVALTRAYHRALEASVARNFPDNGCISCMCHNTDMLYS
AKQTAVVR
ASDDFYPRDPASHTVHISSVAYNTLFLGEFMQPDWDMFHSLHP
AAEYHGA
ARAIGGCCP
IVYSDKPGNHNFLLRKVL
LPDGSVLRAQLPGRPTRDCLFSD
PARDGASLLKI
WNMNK
CAGVVGVFC
NCQAG
WCRVAKKTR
IHDEAPGTLTG
SVRAEDVEAI
AQAA
GTGDWG
GEAVV
YAH
RAGEL
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EPRFVIIDDGWQSISFDGYDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLGPNSPYDPNNFTDLILKGIEHEKLRKKREAISSKSSDLAEI
ESKIKKVVKEIDDLFGGEQFSSGEKSEMKESEYGLKAFTKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKIVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKDVVIHSLEYVCDEYGGRVDLA
KVYYEGLTKSIVKNFNGNGMIASMQHNCNDFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVCASFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTVLKIWBNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTVEWDQKEETSHLGKAEEYVYLNQAEELSLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSESPPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGVSDME
IFF

PsSTS-2:

MAPPLNSTTSNLIKTESIFDLSERKFKVKGFLFHDVPENVSFRSFSI
KPSSENAPPSSLLQKVLAWSHGCGFFGFSHETPSDRLMNSLGSFNGKDFLS
IFRFKTTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIEKCFRS
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENPYDLMKEAYIAIR
VHLNSFRLLEEKTIPNLVDKFGWCTWDAYLTVNPIGIFHGLDDFSKGGV
EPRFVIIDDGWQSISFDGCDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLGPNSPYDPKKFTDLILKGIEHEKLRKKREAISSKSSDLAEI
ESKIKKVVKEIDDLFGGEQFSSVEKSEMKESEYGLKAFTKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKFVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKDVVIHSLEYVCDEYGGRVDLA
KVYYEGLTKSIVKNFNGNGMIASMQQCNDFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFKSDHVCASFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN
PLFDHTLLKIWBNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTQVEWDQKEETSHFGKAEEYVYLNQAEELCLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSESPPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGV

SaSTS:

MAPPNDPISSIFSPLISVKKDNAFELVGGKLSVKNVPLLSEIPSNTFKS
FSSICQSSGAPAPLYNRAQSLSNCGGFLGSQKESADSVTNSLGKFTNRE
FVSIFRFKTTWWSTQWVGTSGSDIQMETQWIMLNPEIKSYAVVIPIVEGK
FRSALFPGKDGHVLI SAESGSTCVKTSFTSIAYVHFSNDPYTLMKDGYT
AVRVHLDTFKLIEEKSAPPLVNKFGWCTWDAYLTVEPAGIWNGVKEFSD
GGFSRFLIIDDGWQSINIDGQDPNEDAKNLVLGGTQMTARLHRFDECEK
FRKYKGGSMMGPKVYFDPKKPKLLISKAIEIEGVKARDKAIQSGITDL

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 L R T N F K G L D D I Y V W H A L A G A W G G V K P G A T H L N A K I E P C K L S P G L D G T M T D
 L A V V K I L E G S I G L V H P D Q A E D F Y D S M H S Y L S K V G I T G V K V D V I H T L E Y V S
 E N Y G G R V E L G K A Y Y K G L S K S L K K N F N G S G L I S S M Q Q C N D F F L L G T E Q I S M
 G R V G D D F W F Q D P N G D P M G V F W L Q G V H M I H C A Y N S M W M G Q I I H P D W D M F Q S
 D H C S A K F H A G S R A I C G G P V Y V S D S L G G H D F D L L K K L V F N D G T I P K C I H F A
 L P T R D C L F K N P L F D S K T I L K I W N F N K Y G G V V G A F N C Q G A G W D P K E Q R I K G
 Y S E C Y K P L S G S V H V S D I E W D Q K V E A T K M G E A E E Y A V Y L T E S E K L L T T P E
 S D P I P F T L K S T T F E I F S F V P I K K L G Q G V K F A P I G L T N L F N S G G T I Q G V V Y
 D E G V A K I E V K G D G K F L A Y S S S V P K R S Y L N G E E V E Y K W S G N G K V E V D V P W Y
 E E C G G I S N I T F V F

VaSTS:

M A P P N D P V N A T L G L E P S E K V F D L S D G K L T V K G V V L L S H V P E N V T F S S F S S
 I C V P R D A P S S I L Q R V T A A S H K G G F L G F S H V S P S D R L I N S L G S F R G R N F L S
 I F R F K T W W S T Q W V G N S G S D L Q M E T Q W I L I E V P E T E S Y V V I I P I I E K S F R S
 A L H P G S D D H V K I C A E S G S T Q V R A S S F G A I A Y V H V A E T P Y N L M R E A Y S A L R
 V H L D S F R L L E E K T V P R I V D K F G W C T W D A F Y L T V N P V G V W H G L K D F S E G G V
 A P R F V V I D D G W Q S V N F D D E D P N E D A K N L V L G G E Q M T A R L H R F E E G D K F R K
 Y Q K G L L L G P N A P S F N P E T I K E L I S K G I E A E H L G K Q A A A I S A G G S D L A E I E
 L M I V K V R E E I D D L F G G K G K E S N E S G G C C C K A A E C G G M K D F T T D L R T E F K G
 L D D V Y V W H A L C G G W G G V R P G T T H L D S K I I P C K L S P G L V G T M K D L A V D K I V
 E G S I G L V H P H Q A N D L Y D S M H S Y L A Q T G V T G V K I D V I H S L E Y V C E E Y G G R V
 E I A K A Y Y D G L T N S I I K N F N G S G I I A S M Q Q C N D F F L G T K Q I P F G R V G D D F
 W F Q D P N G D P M G V F W L Q G V H M I H C S Y N S L W M G Q I I Q P D W D M F Q S D H E C A K F
 H A G S R A I C G G P V Y V S D S V G S H D F D L I K K L V F P D G T V P K C I Y F P L P T R D C L
 F R N P L F D Q K T V L K I W N F N K Y G G V I G A F N C Q G A G W D P K G K K F K G F P E C Y K A
 I S C T V H V T E V E W D Q K K E A E H M G K A E E Y V V Y L N Q A E V L H L M T P V S E P L Q L T
 I Q P S T F E L Y N F V P V E K L G S S N I K F A P I G L T N M F N S G G T I Q E L E Y I E K D V K
 V K V K G G G R F L A Y S T Q S P K K F Q L N G S D A A F Q W L P D G K L T L N L A W I E E N D G V
 S D L A I F F

The calculated overall sequence homologies (%) are shown in Table 2 attached hereto. The homologies between RFSs and SIP are less than 40%. The homologies between RFSs and STSs are not higher than 45%. On the other hand, the homologies among RFSs are all 50% or higher. Thus, the homologies among RFSs are higher than those homologies between RFSs and SIP and between RFSs and STSs.

A molecular phylogenetic tree of the RFSs, SIP and STSs shown in Table 1 is

drawn in Figure 1 attached hereto. The molecular phylogenetic tree is drawn by the UPGMA method using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. In the molecular phylogenetic tree, RFSs, SIP and STSs form different groups respectively.

In summary, Table 2 and Figure 1 show that RFSs, SIP and STSs can be distinguished from one another based upon a comparison of their amino acid sequences.

2) Attached Table 3 shows the identities obtained using the BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. Among Sc-02, Sc-03, Sc-04 and Sc-05, the identities were obtained by searching the "patent database" provided by NCBI (National Center for Biotechnology Information) with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein query vs. translated database (tblastn)" of the NCBI BLAST program. Also, other identities were obtained by searching the "non-redundant database" provided by NCBI with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein-protein BLAST (blastp)" of the NCBI BLAST program. The above-identified amino acid sequences of the RFSs, SIP and STSs are used as the "query" except that the amino acid sequence of Sc-04 used as the "query" is as follows:

Sc-04 (full-length) :

MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT
PSPIDAKSSKNNEDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR
FKVWWTTHWVGNSNGHELEHETQMMLLDKNDQLGRPFVILPILQASFRAS
LQPGLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATVVRM
HLGTFKLLEEKTAPEVIIDKFGWCTWDAYLKVHPSGVWEGVKGLVEGGCP
PGMVLIDDGWQAICHDEDPTIDQEGMKRTSAGEQMPCRLVKLEENYKFRQ
YCSGKDSEKGGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ
AKVVTPKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLYEGLHSRLES
AGIDGVKVDVIHLLEMLSEEVGGRVELAKAYYKALTASVKKHFKGNGVIA
SMEHCNDFFLLGTEAIALGRVGDDFWCTDPGDPNGTYWLQGCHMVHCAY
NSLWMGNFIQPDWDMFQSTHPCAEFHAASRAISGGPVYVSDCVGKHNFKL
LKSLALPDGTILRCQHYALPTRDCLFEDPLHDGKMLKIWNLNKYTGVLG

LFNCQGGGWCPVRRNKSASEFSQTVTCLASPQDIEWNGKSPICIKGMN
VFAVYLFKDHLKLMKASEKLEVSLEPFTFELLTVSPVIVLSKKLIQFAP
IGLVNMLNTGGAIQSMEFDNHIDVVKIGVRGCGEMKVFASEKPVSCLDG
VVVKFDYEDKMLRVQVPWPSASKLSMVEFLF

As shown in Table 3, the identities between RFSs and SIPs are about 40%. The identities between RFSs and STSs range from about 40% to about 50%. On the other hand the identities among RFSs are 60% or higher. The identities among STSs are also 60% or higher. That is, the identities among RFSs or the identities among STSs are higher than the identities between RFSs and SIP or the identities between RFSs and STSs. Thus, RFSs, SIP or STSs can be distinguished based on the results of analysis using BLAST program.

3) Attached Table 4 shows the identities obtained using another BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. All possible pair-wised amino acid sequence comparison were made by the "Blast 2 Sequences" program from NCBI (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>). Sequence identities were calculated using default parameters, program; blastp, matrix; BLOSUM62, open gap penalty; 11, extension gap penalty; 1, gap x_dropoff; 50, expect; 10.0, and word size; 3. The amino acid sequences of the RFSs, SIP and STSs used to calculate sequence identities are identical to those used as the "query" to obtain identities shown in Table 3. Results were essentially the same with former two types of comparison.

4) In conclusion, raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) were clearly distinguished from one another based on comparison of their amino acid sequences.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

This 6th day of September, 2005



Akitsu NAGASAWA

Table 1

| Code | Protein* | Organism | Accession** | Reference | Author/Assignee |
|---------|----------|-----------------------------|-------------|----------------|-------------------|
| Sc-03 | RFS | <i>Beta vulgaris</i> | E37133 | 09/301,766 | Sumitomo Chemical |
| Sc-05 | RFS | <i>Brassica juncea</i> | E36417 | 09/301,766 | Sumitomo Chemical |
| Sc-02 | RFS | <i>Vicia faba</i> | E24423 | 08/992,914 | Sumitomo Chemical |
| Sc-04 | RFS | <i>Glycine max</i> | E24424 | 08/992,914 | Sumitomo Chemical |
| Aj-05 | RFS | <i>Cucumis sativus</i> | AF073744 | Family GH36*** | Ohsumi et al. |
| PsRFS | RFS | <i>Pisum sativum</i> | AJ426475 | Family GH36 | Peterbauer et al. |
| HvSIP | SIP | <i>Hordeum vulgare</i> | M77475 | Family GH36 | Heck et al. |
| PsSTS-1 | STS | <i>Pisum sativum</i> | AJ311087 | Family GH36 | Peterbauer et al. |
| PsSTS-2 | STS | <i>Pisum sativum</i> | AJ512932 | Family GH36 | Peterbauer et al. |
| VaSTS | STS | <i>Vigna angularis</i> | Y19024 | Family GH36 | Peterbauer et al. |
| AmSTS | STS | <i>Alonsoa meridionalis</i> | AJ487030 | Family GH36 | Voitsekhovskaja |
| SsSTS | STS | <i>Stachys affinis</i> | AJ344091 | Family GH36 | Pesch and Schmitz |

*Protein: RFS, Raffinose synthase; SIP, Seed Imbibition Protein; STS, Stachyose synthase.

** Accession: GenBank Accession Number.

***Family GH36: glycoside hydrolase family 36 (see Carbohydrate-Active Enzymes (CAZy) database: http://afmb.cnrs-mrs.fr/CAZY/GH_36.html)

Table 2

Table 3

Table 4

Fig. 1

[GENETYX : Evolutionary tree]
Date : 2004.2.4
Method: UPGMA

